Chapter 2

Clinical Aspects of *WT1* and the Kidney

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Abstract

For more than 30 years, *WT1* mutations have been associated with complex developmental syndromes involving the kidney. Acting as a transcription factor, *WT1* is expressed throughout the nephron and controls the reciprocal interactions and phenotypic changes required for normal renal development. In the adult, WT1 expression remains extremely high in the renal podocyte, and at a lower level in the parietal epithelial cells. Wt1-null mice are unable to form kidneys [1]. Unsurprisingly, *WT1* mutations lead to significant abnormalities of the renal and genitourinary tract, causing a number of human diseases including syndromes such as Denys–Drash syndrome, Frasier syndrome, and WAGR syndrome. Recent methodological advances have improved the identification of *WT1* mutations, highlighting its importance even in nonsyndromic renal disease, particularly in steroid-resistant nephrotic syndrome. This vast spectrum of *WT1*-related disease typifies the varied and complex activity of WT1 in development, disease, and tissue maintenance.

Key words Renal podocyte, Nephrotic syndrome, Glomerular sclerosis, Mesangial cells, Genitourinary syndromes, Genetic testing, Transplantation

1 WAGR Syndrome

The first *WT1*-associated disease to be identified was WAGR syndrome (Wilms' tumor, Aniridia, Genitourinary malformation, and mental Retardation), caused by deletions of 11p13, and the loss of both *WT1* and *Pax6* [2]. Approximately 50% of WAGR patients develop Wilms' tumor [2, 3] and the renal prognosis for patients both with and without Wilms' tumor is poor, with a cumulative risk of renal failure ranging from 40 to 60% by the age of 20 [4]. The most common pathological finding is focal segmental sclerosis (FSGS), originally attributed to glomerular hyperfiltration, as a consequence of reduced nephron mass following nephrectomy. However, proteinuria and nephrotic syndrome, with FSGS on renal biopsy, have been identified in WAGR patients without Wilms' tumor, indicating complete loss of one *WT1* allele alone may induce FSGS [5]. Animal models of the renal phenotype, such as the *Wt1*-heterozygous mouse, support this conclusion, as glomerular sclerosis develops in this model with aging [6, 7].

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2 Denys–Drash Syndrome

Denys–Drash syndrome (DDS) is the best characterized *WT1*-related disorder. The classic syndrome consists of Wilms' tumor, rapidly progressive glomerular disease, and genitourinary abnormalities, particularly male pseudohermaphroditism. The characteristic renal disease is diffuse mesangial sclerosis, assumed to be a developmental or paracrine effect, as WT1 is not expressed in mature mesangial cells. DDS tends to be associated with mutations in the DNA-binding domains of *WT1*. This is thought to affect transcription factor activity, and the profound phenotype may result from the mutant protein acting in a dominant negative manner, as cell lines expressing DDS mutations abolish binding to known WT1-binding targets [8].

A number of animal models of DDS have been developed, which partially recapitulate the human disease, although only one Wilms' tumor has ever been described [9, 10]. These, in combination with conditionally immortalized DDS podocytes in culture, provide evidence of an abnormal podocyte phenotype in DDS. This is unsurprising given the continued high expression of WT1 in the adult podocyte, and profound podocyte damage following conditional Wt1 loss [11]. Histology and immunohistochemical analysis of DDS kidneys demonstrates podocytes are hypertrophied and effaced, with areas of continued proliferation, especially where WT1 expression is lowest. Overexpression of PDGFa and TGFB in these areas is consistent with a pathological epithelial to mesenchymal transition [12]. More recently, evidence has emerged indicating that podocytes in DDS resemble an earlier developmental stage, having not differentiated fully. DDS podocytes continue to express the stimulatory form of VEGF-A (VEGF165), which is normally only expressed from the S-shaped body stage of nephron development, and stimulates glomerular endothelial cell proliferation, migration, and differentiation [13]. Upon differentiation, mature podocytes begin to express an inhibitory form of VEGF (VEGF165b). Using semi-quantitative PCR to analyze human biopsy specimens, this is completely lacking in DDS podocytes [14]. This study also revealed that glomerular basement membrane constituents in DDS patients also resemble those at the S-shaped body developmental stage, with high levels of collagen α1(IV) and laminin β1 and a relative lack of collagen α4(IV) and laminin β2, which are normally found in normal adult glomeruli. The authors interpret this to mean that DDS podocytes are halted in development and do not proceed fully through differentiation. The paracrine influence of abnormal podocytes and an impaired glomerular filtration barrier would explain the profound nephrosis and diffuse mesangial sclerosis in these patients.

3 Frasier Syndrome

Frasier syndrome (FS) describes the combination of male pseudohermaphroditism, predisposition to gonadoblastoma and glomerular disease (usually FSGS), which causes renal failure within the first two decades of life. It is usually caused by point mutations affecting the splice sites in exon 9 of the *WT1* gene. The risk of Wilms' tumor is much lower than in DDS [15, 16]. Splice site mutations lead to an imbalance in the expression of the *WT1*-KTS isoforms, with relative underexpression of the +KTS isoform. This is known to play a role post-transcriptionally as a binding partner for splicing factors and is located in splicing speckles [17–19]. Mouse models unable to express either the +KTS or –KTS isoform have differing renal phenotypes, although neither is as severe as the *Wt1*-null mouse, implying a degree of redundancy is present [17, 18]

4 Meacham Syndrome

Meacham syndrome describes a rare and complex multimalformation syndrome reminiscent of the *Wt1*-null mouse. The phenotype includes male pseudohermaphroditism, abnormal female internal genitalia, complex congenital heart defects, and diaphragmatic hernia [20]. *WT1* mutations have been found in a number of cases, affecting the DNA-binding zinc finger regions and including mutations previously described in DDS.

Importantly, these genotype-phenotype correlations are not clear-cut. The mutations described are generally associated with their relevant syndromes, but exceptions exist. Intron 9 splice site mutations have been described in patients with clinical DDS but without Wilms' tumor [21], and intron 9 mutations which should not have affected splicing have been found in patients with clinical Frasier syndrome [22]. The influence of other modifying genes or environmental factors remains a subject for further research.

Stronger correlation between genotype and phenotype is found with regard to risk from Wilms' tumor. A recent paper reviewed over 50 patients with nephrotic syndrome. Those with missense or nonsense mutations, as is usually found in DDS, were found to have a high risk of Wilms' tumor, whereas splice mutations, as seen in patients with Frasier Syndrome, had a very low risk [23]. The mechanisms explaining this difference are not clear, but are attributed to the potential dominant negative effect of DDS mutant protein.

5 Nonsyndromic Renal Disease

Advances in genetic technology and sequencing techniques have led to an increased recognition of the role of *WT1* mutations in nonsyndromic renal disease. *WT1* mutations have been found to cause up to 12% of SRNS in children and young adults, particularly in phenotypic females. This is on par with the most common genetic cause of SRNS, *NPHS2* (podocin) mutations, which account for up to 26% of cases [24].

Given its complexity, screening for *WT1* and other genetic mutations in SRNS has been difficult. Traditional Sanger sequencing techniques were time-consuming and expensive, particularly as there exists significant genetic heterogeneity and phenotypic variability amongst the various causes of SRNS. Even limiting genetic analysis to the most frequently mutated causative genes remained expensive, and therefore missed unusual and/or multiple mutations [25].

Next Generation sequencing allows for increased throughput and decreased cost, thus facilitating the cost-effective and simultaneous analysis of multiple genes. This has led to the identification of novel genes associated with SRNS as well as expanding the genetic heterogeneity of the condition. *WT1*-associated renal diseases are no exception, with the identification of a familial form of incomplete DDS due to a classic exon 9 DDS mutation (1180C>T: R394W). An unaffected father passed the mutation to his son and daughter [26]. This family demonstrated a DDS-like phenotype in the son, FS-like phenotype in the daughter, and an unaffected father despite all carrying the same classic DDS mutation. Widespread application of NGS techniques to allow simultaneous analysis of multiple genes may provide answers as to the phenotypic variability demonstrated in *WT1*-related disorders by identifying novel genetic variants and disease-modifying alleles [23].

Nonsyndromic *WT1* mutations have also been identified as a cause of inherited autosomal dominant FSGS. Whole exome and direct sequencing techniques were used to investigate the cause of AD FSGS in two northern-European families. A nonsynonymous heterozygous missense change in exon 9 (1327G>A; R458Q) was identified. Unlike WT mRNA, R458Q mRNA was unable to rescue zebrafish morpholinos unable to express Wt1a (one of the two zebrafish Wt1 orthologs) and expression of WT1R458Q in HEK293 cells reduced the expression of podocyte-specific genes including nephrin, synaptopodin, and CD2AP relative to wild-type WT1, consistent with an abnormal podocyte phenotype leading to FSGS [27].

This is the first paper to demonstrate potentially direct regulation of synaptopodin expression by WT1. However, efficacious treatment of SRNS caused by *WT1* mutations has been demonstrated via the use of cyclosporin. This is not thought to be via its immunosuppressive effect, but via prevention of synaptopodin degradation, and thus stabilization of the podocyte cytoskeleton [28].

Nonsyndromic forms of kidney disease have been caused by splice site mutations in intron 9 in 46,XX females (usually associated with Frasier syndrome). Missense mutations in exons 8 and 9 (usually associated with DDS) have also been found in cases of isolated diffuse mesangial sclerosis without Wilms' tumor [29, 30].

From a methodological point of view, these developments have highlighted the importance of genetic testing in steroid resistant nephrotic syndrome in the clinic. As described above, a few cases of nephrotic syndrome caused by *WT1* mutations have been reported which demonstrated a partial response to cyclosporin treatment. In general, genetic causes of nephrotic syndrome do not respond well to immunosuppression so given the toxic side effect profile and lack of efficacy, expert opinion suggests these agents should be avoided or used cautiously [31]. The ability to rapidly screen for *WT1* mutations, and/or specific WT1 mutations that may confer a more favorable risk/benefit profile could offer important therapeutic options to this subgroup of patients. Given the variability of genotype-phenotype correlations, genetic testing also remains vital for other family members when screening for potential organ donors and for the likelihood of disease recurrence in a transplanted graft, and, in theory, for pre-emptive treatment strategies. Although no evidence yet exists for preventative treatment in affected individuals, it is known in other genetic forms of kidney disease that such strategies can be successful, such as the specific use of Angiotensin Converting Enzyme inhibitors in Alport syndrome [32, 33].

6 Conclusion

The importance of *WT1* mutations in developmental renal disorders has been long recognized. Novel genetic technologies, particularly rapid sequencing techniques, have allowed the identification of far more *WT1* mutations, relevant to clinical practice, and thus highlighting the range of *WT1* disease and variable phenotype-genotype correlation. The cause of these profound differences and the wide range of phenotypes provide a focus for further research into the molecular biology of *WT1* as described in subsequent chapters.

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